observations, and the functionality of the channels in the bead-buttressed unilamellar membrane (bBUM) system was examined by electrical recordings of voltage-gated activities. We anticipate that this novel membrane system will provide a new technique to study how lipids influence membrane proteins.

3060-Pos Board B215
Toward Understanding the Role of Angiomotin Lipid Binding in Cellular Proliferation and Migration
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The Angiomotin (Amot) family of adaptor proteins directly integrates the signaling that controls cellular differentiation and cell growth. Amot family members bind core polarity proteins that control the organization of the apical domain of epithelial cells as well as Yap, a transcriptional co-activator that appears to be the key regulator of cell growth. A critical feature of all Amot proteins is a novel lipid binding domain, the Amot coiled-coil homology (ACCH) domain, which confers its association with membranes and affects membrane curvature. This domain, while sharing some features of BAR domains, i.e. a predicted coiled coil fold of approximately 240 residues, also has unique properties including the ability to selectively bind monophosphorylated phosphatidylinositols (PI). Similar binding of PI has been reported in other protein domains, including FYVE, PX and PH domains where PI binding is mediated through basic residues within a loop rich in lysines, arginines, as well as hydrophobic amino acids. The ACCH domain is predicted to have a coiled-coil fold and is rich in lysines.
One of our goals is to delineate which amino acids contribute to PI binding, thereby suggesting possible routes to modulate the increased Amot80 ACCH domain activity that is associated with ductal hyperplasia, and later breast cancer. Site-directed mutagenesis was employed to probe the specific contributions of selected lysines and arginines toward lipid head-group binding. The effect of the mutation was then analyzed using liposome sedimentation, FRET, and SAXS to monitor lipid binding. As the ACCH domain lacks tryptophan residues, protein association with liposomes was followed by monitoring changes in protein fluorescence associated with the proximity of tyrosines 11, 47, or 118 to lipid.
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Assembly and Activity of Respiratory Complex II in Nanolipoprotein Particles
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Mitochondrial respiratory Complex II (succinate:ubiquinone oxidoreductase) couples the citric acid cycle to the electron transport chain by oxidizing succinate in the matrix and passing electrons to ubiquinone in the inner membrane. Complex II is comprised of a soluble catalytic heterodimer (Sdh1/Sdh2) and a membrane-bound heterodimer (Sdh3/Sdh4). The structure of Complex II is well established; however, little is known about how the lipid environment regulates holocomplex assembly and activity. To address this question, we reconstituted Complex II from native biomembranes into nanoscale phospholipid bilayers (nanodiscs) containing a defined lipid content. We found that the dimeric phospholipid cardiolipin, the signature lipid of energy-conserving membranes, is critical for Complex II stability and function. First, the presence of cardiolipin in the bilayer promoted the interaction of the soluble and membrane-bound dimers. Second, cardiolipin was essential for enzymatic activity of the reconstituted complex and for curtailment of reactive oxygen species production. The function of cardiolipin could be partially compensated by the presence of phosphatidylglycerol, another phospholipid with an anionic headgroup; moreover, reducing the acyl chain lengths of cardiolipin used for reconstitution prevented its stimulatory effect on Complex II activity. Hence, both the headgroup and hydrocarbon chains of cardiolipin play important roles. Using this experimental platform, we have employed site-specific fluorescence labeling to address which structural elements of Complex II membrane subunits undergo conformational dynamics during assembly. Our results indicate that Sdh3 matrix-facing helix I, which interacts extensively with the catalytic dimer in the holocomplex, undergoes structural changes when reconstituted with Sdh4, but not Sdh4 homologs, suggesting that this helix may act as a conformational switch for downstream assembly steps (e.g., recruitment of the soluble dimer). Taken together, we show that Complex II is structurally dynamic during assembly and that its function is highly lipid-dependent.

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Purification and Characterization of the Tetrameric Potassium Channel Kcsa in "Native Nanodiscs"
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We explored a new discovery in membrane research of amphipathic copolymers of styrene and maleic acid (SMA) that have the unique potential to directly extract proteins from membranes in the form of "native nanodiscs" without the need for detergent [1,2]. *E-coli* cells overexpressing a His-tagged version of the potassium channel KcsA were incubated with SMA and the conditions were optimized for extraction of the protein. After solubilization of the membrane, we found that KcsA indeed could be purified on a Ni^{2+}-NTA column in the form of nano-sized discs, as was confirmed by negative stain transmission electron microscopy (TEM) experiments. SDS-PAGE analysis showed that the protein in these nanodiscs is present as a tetramer, running at the same position as when subjected to SDS-PAGE from its native membrane or after purification in detergent.
Presently we are comparing the stability of this tetramer in the nanodiscs with that of purified KcsA in *n*-dodecyl β-D-maltoside (DDM) micelles and KcsA in native membranes. This is done by comparing the effects of heat-incubation and exposure to small alcohols using gel shift assays. Furthermore, to gain information about possible preferential lipid interactions of KcsA, the lipid composition of the purified, KcsA containing nanodiscs is being analyzed and compared to that of the total lipid composition in *E-coli*. Results of these studies will be presented.
1) Knowles TJ, Finka R, Smith C, Lin Y-P, Dafforn T, Overduin M (2009) J Am Chem Soc 131, 7484-7485
2) Orwich-Rydmark M, Lovett JE, Lindholm L, Graziadei A , Hicks M, Watts A (2012) Nanoletters 12, 4687-4692

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Determining the Role of NS4B in Membrane Remodelling during Hcv Replication
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Hepatitis C is an RNA virus that replicates in association with intracellular membranous structures call membranous webs (MWs). Viral protein NS4B is a key organizer of replication, one crucial function being the induction of MWs. The mechanisms of MW formation are unknown, but it clearly involves induction of membrane curvature, which may require NS4B oligomerisation and possibly hydrophobic wedging. NS4B is known to oligomerise, and the N-terminal amphipathic helix AH2 has been implied as a major determinant of self-association. In order to understand the process of MW induction, we aimed to determine AH2's capacity to remodel membranes by studying the interaction of AH2 with membranes mimicking those found within the cell using 2H and 31P solid-state NMR. Our results show changes in membrane morphology, induced by AH2 in negatively charged vesicles, an effect not observed in neutral bilayers indicating a requirement for negatively charged lipids. Chemical cross-linking studies of AH2 in lipids vesicles confirms AH2 homo-oligomerisation and suggests a charge dependency; with larger oligomers observed in neutral lipid bilayers compared to negatively charged lipid bilayers and lipid mixtures mimicking cellular membranes. These results suggest that AH2 plays a crucial role in NS4B's capacity to alter membrane morphology.

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Phosphorylation of the Amphipathic Helix Changes the Lipid Binding Capacities of PICK1
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PICK1 (Protein Interacting with C-kinase 1) is a functionally important protein, which is distributed mainly in testis, pancreas and brain. It has been shown to play a central role in regulation of dense core vesicles from the golgi apparatus and trafficking of ionotropic glutamate receptors.
PICK1 contains a N-terminal PDZ-domain, which we have earlier demonstrated to be important for interaction with a large number of proteins, including several important receptors and transporters. In addition, it has a BAR (Bin/Amphiphysin/Rvs) domain in the C-terminal end. BAR domains are generally believed to either recognize or induce curvatures of lipid membranes, but as we have demonstrated, proteins of the N-BAR family (incl. PICK1) binds lipids and recognizes membrane curvature (MC) through an associated amphipathic helix (AH) rather than through the BAR domain itself.
Here we show that the lipid binding AH of PICK1 contains a phosphorylation-site, which, through PKC activation, is responsible for an altered cellular